ELSEVIER

www.elsevier.com/locate/carres

Carbohydrate Research 334 (2001) 97–103

CARBOHYDRATE RESEARCH

Synthesis of the milk oligosaccharide 2′-fucosyllactose using recombinant bacterial enzymes

Christoph Albermann, Wolfgang Piepersberg, Udo F. Wehmeier*

Institut für chemische Mikrobiologie, Bergische Universität GH, FB9, Gauss-Strasse 20, D-42097 Wuppertal, Germany

Received 18 April 2001; accepted 29 May 2001

Abstract

The enzymatic synthesis of GDP-β-L-fucose and its enzymatic transfer reaction using recombinant enzymes from bacterial sources was examined. The GDP-D-mannose 4,6-dehydratase and the GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase-4-reductase from *Escherichia coli* K-12, respectively, were used to catalyse the conversion of GDP-α-D-mannose to GDP-β-L-fucose with 78% yield. For the transfer of the L-fucose to an acceptor, we cloned and overproduced the α-(1→2)-fucosyltransferase (FucT2) protein from *Helicobacter pylori*. We were able to synthesise 2′-fucosyllactose using the overproduced FucT2 enzyme, enzymatically synthesised GDP-L-fucose and lactose. The isolation of 2′-fucosyllactose was accomplished by anion-exchange chromatography and gel filtration to give 65% yield.

Keywords: Bacterial α-(1→2)-fucosyltransferase; GDP-L-fucose; 2′-Fucosyllactose; Enzymatic synthesis; *Helicobacter pylori*, *Escherichia coli*

1. Introduction

Oligosaccharides mediate inter- and intracellular communication processes, e.g., cell–cell recognition, tumour development; they are ligands for pathogens and modulate the functions of hormones and antibiotics.[1,2] Their multiple functions, as well as their enormous structural variety, has stimulated interest in the pathways leading to oligosaccharides and their analogues. The availability of these molecules allows new insights into their biological functions and might lead to new carbohydrate-based therapeutics.[3] Oligosaccharides are generally synthesised in vivo by glycosyltransferases that transfer a single nucleotide activated sugar to an acceptor or a growing carbohydrate chain. Glycosyltransferases show high regio- and stereoselectivity and there is no need for chemical protection or deprotection of the substrate as one would need in chemical synthesis. One disadvantage of the enzymatic synthesis of oligosaccharides is the limited availability of the glycosyl donor and a limited substrate tolerance of the enzymes. The substrate specificity of the glycosyltransferases can be improved or changed in order to create new enzymes with new specificity for unnatural acceptor and/or donor substrates. Non-natural and new oligosaccharides could then be synthesised by using a modified sugar that is transferred by glycosyltransferases with broader or engineered substrate specificity or by using a non-natural acceptor for the transferred monosaccharide.[4]

* Corresponding author. Tel.: +49-202-4392594; fax: +49-202-4392698.

E-mail address: wehmeier@uni-wuppertal.de (U.F. Wehmeier).

0008-6215/01/$ - see front matter
PII: S0008-6215(01)00177-X

Fucosylation is the terminal and critical step in the in vivo synthesis of many biologically important oligosaccharide side chains in glycolipids and glycoproteins.[5,6] Several fucosyltransferases from prokaryotes and eukaryotes have been cloned and studied. Many fucosyltransferases have been studied because they are involved in the biosynthesis of the antigens LewisX (Le^X) and LewisY (Le^Y).[4,7,8] Lewis antigens are also parts of the lipopolysaccharide (LPS) of *Helicobacter pylori*. The expression of Lewis antigens by *H. pylori* is thought to provide a way to overcome the detection by the human immune system, because these antigens are also expressed by the human gastric epithelium.[8] One fucosylated substrate of commercial interest is 2′-fucosyllactose, which has been detected, in human milk. Like many other oligosaccharides, 2′-fucosyllactose is believed to protect infants from infectious agents.[9] 2′-Fucosyllactose is synthesised in vivo by a fucosylation reaction catalysed by the human α-(1→2) fucosyltransferase (E.C.2.4.1.69). The donor substrate in this glycosyltransferase reaction is GDP-L-fucose that is transferred to lactose. In the genome of *H. pylori*, several genes encoding putative fucosyltransferases have been found and among them one gene encoding a α-(1→2)-fucosyltransferase (FucT2[7]). This enzyme catalyses the transfer of GDP-L-fucose to a galactosyl-residue of Le^X during the Le^Y biosynthesis.[7,8] Therefore, we studied whether the *H. pylori* FucT2 may serve as a tool for the biosynthesis of 2′-fucosyllactose.

In this report, we describe the cloning and the overproduction of the α-(1→2) fucosyltransferase FucT2 from *H. pylori* NCTC364. The recombinant protein was tested for transfer activities on a number of acceptor substrates, including lactose. Using the *H. pylori* α-(1→2) fucosyltransferase, enzymatically synthesised GDP-L-fucose[10] and lactose, we were able to synthesise 2′-fucosyllactose (α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose). The structure of 2′-fucosyllactose was confirmed by ^{13}C, ^{1}H and related 2D NMR spectra.

2. Results and discussion

The chemical synthesis of GDP-L-fucose along classical routes had been described previously.[11,12] A novel way to GDP-L-fucose is the use of recombinant enzymes.[10] Starting from GDP-D-mannose, which can also be prepared by enzymatic synthesis,[13,14] the intermediate GDP-4-keto-6-deoxymannose was formed by the GDP-mannose 4,6-dehydratase in the presence of the cofactor $NADP^+$ (Fig. 1). In a second step, GDP-4-keto-6-deoxymannose was converted into GDP-L-fucose by the bifunctional GDP-fucose-synthetase[15] in the presence of the co-substrate NADPH (Fig. 1). A one-pot reaction of both enzymatic steps is not efficient because a strong feedback inhi-

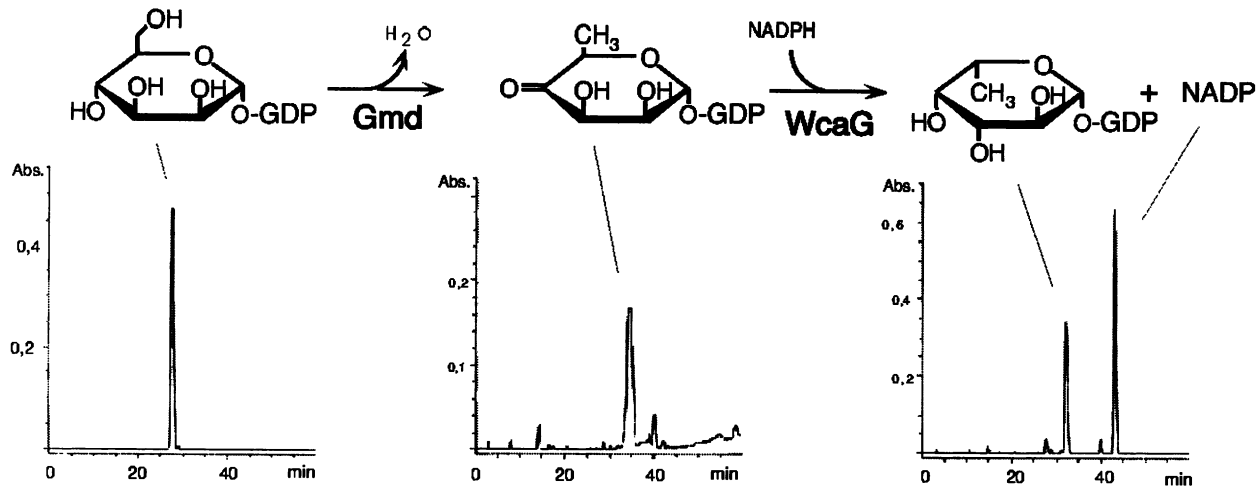

Fig. 1. HPLC analysis of nucleotide-activated sugars (from left to right: GDP-D-mannose, GDP-4-keto-6-deoxymannose and GDP-L-fucose) of the reaction mixture. Gmd, GDP-mannose 4,6-dehydratase; WcaG, GDP-fucose synthetase.

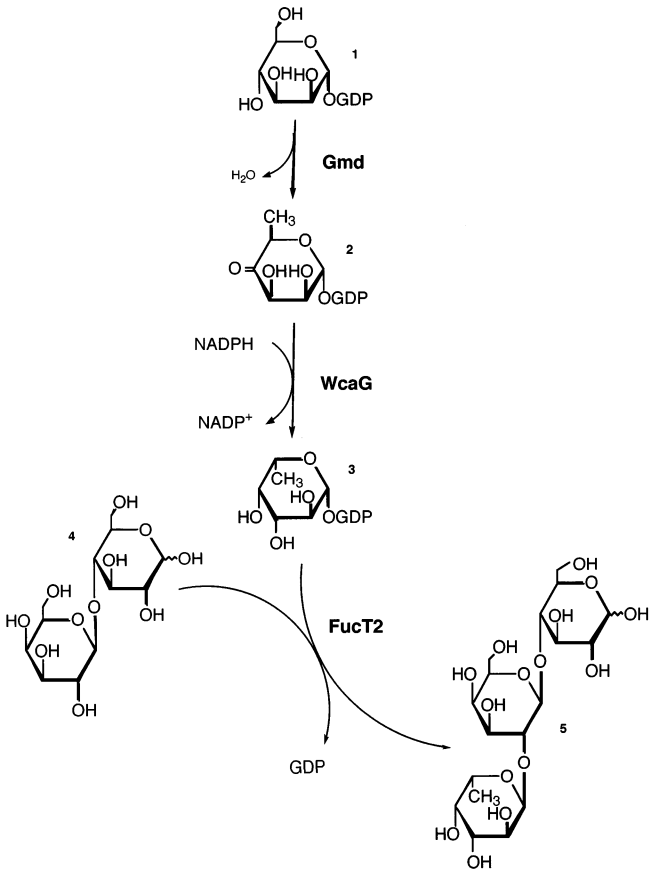

Fig. 2. Enzymatic synthesis of 2′-fucosyllactose. 1, GDP-D-mannose; 2, GDP-4-keto-6-deoxymannose; 3, GDP-L-fucose; 4, lactose; 5, 2′-fucosyllactose. The enzymes used for the synthesis are bold. GDP, guanosine 5′-diphosphate; $NADP^+$, nicotinamide–adenine–dinucleotidphosphate; NADPH reduced $NADP^+$.

bition of the GDP-mannose 4,6-dehydratase by GDP-L-fucose ($IC_{50} = 10$ μmol) had been detected.[16] For the purification of GDP-L-fucose, a preparative HPLC with C-18 column was performed, as well as anion exchange chromatography, to obtain the sodium-salt and a final gel filtration. The obtained activated-deoxyhexose was isolated in 78% yield and the data of the ^1H, $^1H^1H$-COSY, ^{13}C and ^{31}P NMR spectroscopy were in good accordance with those from the chemically synthesised GDP-β-L-fucose.[11,12] Recently, an alternative synthesis pathway for GDP-L-fucose using the combination of recombinant *Escherichia coli* cells overexpressing GDP-L-fucose biosynthetic genes and *Corynebacterium ammoniagenes* cells has been described.[28] This method allowed the production of 18.4 g/L GDP-L-fucose.

The gene *fucT2* encoding the α-(1→2) fucosyltransferase from *H. pylori* NCTC 364 was cloned and overexpressed in *E. coli*. The recombinant FucT2 protein was tested for transfer-activity using lactose as an acceptor (Fig. 2). The expression of *fucT2* cloned in *E. coli* JM109 (DE3) pCAW36 or pCAW40, respectively, resulted in very low amounts of soluble protein under standard conditions. In crude extracts, no fucosyltransferase-activity with the substrate lactose was observed. An overproduction of an active FucT2 (0.02 nkat/mg protein) in *E. coli* JM109 (DE3) pCAW36 or pCAW40 was possible only at lower temperature and in betain-sorbitol-LB-medium, however $\geq 90\%$ of the protein was insoluble. The expression of the FucT2 as a glutathione-*S*-transferase fusion protein (GST-FucT2) under the control of the *lac*-promotor in pCAW53 reduced the amount of inclusion bodies and consequently led to a higher specific activity (0.12 nkat/mg protein). This fusion protein was enriched by an affinity chromatography on a gluthathione–sepharose column (1.5 nkat/mg protein).

The transfer of the L-fucose to various acceptor molecules was determined. The GST-FucT2 protein catalysed the transfer of L-fucose to the acceptor molecules Gal-β-(1→3)-GlcNAc-R, Gal-β-(1→4)-GlcNAc-R, Gal-β-(1→4)-Glc-R and also to PNP-β-Gal. A transfer to the acceptor molecules Gal-β-(1→3)-[α-(1→3)-Fuc]-GlcNAc-R and Gal-β-(1→4)-[α-(1→3)-Fuc]-GlcNAc-R had been shown before.[7]

The enzymatic synthesis of fucosyllactose was carried out with the GST-FucT2 fusion protein under the conditions described in Section 3. The consumption of GDP-L-fucose was monitored by HPLC. All of the GDP-L-fucose was consumed after 7.5 h. By anion-exchange chromatography, GDP and GMP were removed and after gel filtration the desired 2′-fucosyllactose (α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose) was obtained in 65% yield. The structure was identified by ^{13}C, ^1H and related 2D NMR spectra. The kinetic data for the fucosylation of the unnatural substrate lactose were determined using radioactively labelled lactose and GDP-L-fucose. These experiments indicated that the apparent K_m of the donor

GDP-L-fucose is 32 μM and the K_m of the acceptor lactose is 168 μM, analysed by the concentration of labelled 2′-fucosyllactose. Fucosylation assays were also performed using some other bacterial fucosyltransferases. The corresponding genes had been cloned and overexpressed similar to FucT2 from *H. pylori*. All these fucosyltransferases (WbcH from *Yersinia enterocolitica* O8, FucT2 and FucT3 from *Vibrio cholerae* O22, WcfB from *Bacterioides fragilis* were tested under the same conditions and none of them accepted lactose as a substrate (data not shown).

In summary, we have shown that 2′-fucosyllactose can be synthesised enzymatically using the *H. pylori* FucT2 enzyme that utilises a broad range of acceptors. This enzyme was the only enzyme that accepted lactose as a substrate for fucosylation. All other bacterial fucosyltransferases tested so far did not accept lactose as a substrate. The specificity of the donor substrate has not been tested yet, but it will be a subject of further investigations. The recombinant fucosyltransferase will be readily available in larger quantities after overproduction in a bacterial host. The use of other glycosyltransferases in similar approaches will lead to new biosynthetic pathways for new and rare oligosaccharides.

3. Experimental

Materials.—All protein standards, antibiotics, isopropyl-thiogalactoside (IPTG), nucleotides, were obtained from Sigma (Deisenhofen, Germany); nucleotide sugars were obtained from Calbiochem (Schwalbach, Germany). All other chemicals used were commercially available and of analytical grade.

Bacterial strains and culture conditions.—The bacterial strains used in this study were *E. coli* DH5α[17] and *E. coli* JM 109 (DE3) (Novagen, USA). Strains of *E. coli* were grown at 37 °C in Luria–Bertani broth (LB)[18] or in M9-medium;[19] for solid media, 18 g/L agar was added. *E. coli* JM 109 (DE3)/pCAW36 or pCAW40 was grown in LB-medium with 1 M sorbitol and 2.5 mM betain at 27 °C. Ampicillin (Amp; 100 μg/mL) was added to the media as a selection marker for the plasmid carrying strains.

Overproduction of GDP-D-mannose 4,6 dehydratase and GDP-L-fucose synthetase.—For the overproduction of Gmd and WcaG,[20] *E. coli* JM109 (DE3) with the recombinant plasmids, pCAW21.1[10] or pCAW22.2[10] was grown in LB-media to an OD_{540} of 0.6. The cells were then induced by IPTG (0.7 mM) for 90 min. Subsequently, the cells were harvested by centrifugation, washed twice in ice-cold 50 mM Tris–HCl buffer pH 7.5, and suspended in extraction buffer [50 mM Tris–HCl buffer pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol]. After the disruption by sonication, the crude extract was clarified by centrifugation at 30,000*g* for 15 min. The crude extracts were analysed by SDS-PAGE.[21]

Overproduction of the α-(1→2) fucosyltransferase.—All DNA manipulations were performed according to the procedures described by Sambrook et al.[19] The *fucT2* gene for α-(1→2) fucosyltransferase from *H. pylori* NCTC 364 was amplified by PCR. The primers were created on basis of the published sequence[22] (FucT2HP 1 5′-GACAAATAAAGGGATCATATGGCTTTTAAGG-3′) (forward direction); FucT2HP 2 5′-CGCTCGCTATAAAGAAATCAGATCTAAAATC-3′) (reverse direction). The products of the PCR amplification, with Vent polymerase (BioLabs, Schwalbach/Taunus), were hydrolysed with *Nde*I and *Bgl*II (Gibco BRL, Eggenstein, Germany) and ligated into the vectors pET11a and pET16b (Novagen, Madison, WI, USA) which were treated with *Nde*I and *Bam*HI. After ligation with T4-DNA-ligase (BioLabs, Schwalbach/Taunus), the recombinant plasmids pET11a/*fucT2* (pCAW40) and pET16b/*fucT2* (pCAW36) were obtained and transformed into *E. coli* DH5α. The insert structure of each recombinant derivative was verified by restriction analysis and DNA-sequencing.[23] For over-expression of the proteins, the recombinant plasmids were transformed into *E. coli* JM 109 (DE3). To create a glutathione-*S*-transferase (GST) fusion protein, the *fucT2*-gene was amplified from pCAW36 using the following primers GST36.8 1 (5′-CAGCGGCCATCTCGAGGGTCGTCATATG-3′) (forward direction), GST36.8 2 (5′-GAAAAGTGCCACCTGAC-

GTCTAAGAAAC-3′) (reverse direction). The PCR product was hydrolysed with *Xho*I and *Aat*II (Gibco BRL, Eggenstein, Germany) and ligated into the vector pGEX4T-1 (Pharmacia, Sweden) which was hydrolysed with *Xho*I and *Aat*II. The recombinant plasmid pGEX4T-1/*fucT2* (pCAW53) was obtained and transformed into *E. coli* DH5α. For the overproduction of the GST-FucT2 protein, *E. coli* DH5α pCAW53 was grown in LB-medium at 37 °C to an OD_{540} of 0.8. Then the cells were induced by IPTG (0.4 mM) for 16 h. Subsequently, the cells were harvested by centrifugation, washed twice in ice-cold 50 mM Tris–HCl buffer, pH 7.5, and suspended in extraction buffer [50 mM Tris–HCl buffer pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol]. After the disruption by a french-press, the crude extract was clarified by centrifugation at 30,000*g* for 15 min. The purification of the GST-FucT2 protein was carried out by affinity chromatography on a GSTrap-column (5 mL, Pharmacia, Sweden) according to the supplier's instructions at 4 °C. The protein-containing fractions were analysed by SDS-PAGE.[21]

*GDP-*D*-mannose 4,6-dehydratase.*—For the measurement of the activity of the GDP-D-mannose 4,6-dehydratase, both a spectroscopic[24,25] and a chromatographic test system were used to estimate the increase of GDP-4-keto-6-deoxy-D-mannose. The standard assay (100 μL) contained 50 mM Tris–HCl buffer, pH 7.5, 10 mM $MgCl_2$, 4 mM GDP-D-mannose, 50 μM $NADP^+$ and different amounts of crude extract with Gmd or His-tag Gmd. The concentration of the protein was determined according to Bradford.[26] The reactions were incubated at 37 °C and samples were measured at different times between 0 and 60 min. For the photometric analysis, the reactions were stopped by adding 950 μL 100 mM NaOH to 50 μL of the reaction mixture, followed by a further incubation for 20 min at 37 °C and then the absorption was measured at 320 nm.

*GDP-4-keto-6-deoxy-*D*-mannose 3,5-epimerase-4-reductase.*—The enzyme activity of the GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase-4-reductase was determined by the consumption of NADPH at 340 nm and 37 °C. The standard assay contained 50 mM Tris–HCl buffer pH 7.5, 2 mM GDP-4-keto-6-deoxy-D-mannose, 10 mM $MgCl_2$ and different amounts of WcaG or His-tag WcaG and 4 mM NADPH. The reaction was started by the addition of different concentrations of GDP-4-keto-6-deoxy-D-mannose (final volume 500 μL).

α-(1→2) Fucosyltransferase.—The activity of the overproduced α-(1→2)-fucosyltransferase from *H. pylori* was assayed in a total volume of 20 μL as follows: 2 mM GDP-L-fucose, 3 mM lactose, 50,000 dpm [D-glucose-1-^{14}C]-lactose, 50 mM Tris–HCl buffer, pH 7.5, 10 mM $MnCl_2$, 100 mM NaCl and different amounts of the recombinant GST-FucT2 protein. Reactions were conducted at 37 °C for up to 60 min and subsequently chromatographed on Silica Gel F_{254}, TLC sheets (E. Merck, Darmstadt, Germany) using 7:6:1 AcOH–$CHCl_3$–water. The sugar components were localised by staining with a solution of 2.5% molybdatophosphoric acid hydrate, 1% ceric sulfate, 6% H_2SO_4 in water. The spots of the radioactively labelled lactose and fucosyllactose were measured in a scintillation counter (Wallac 1415 DSA, Bad Wildbad, Germany). In tests using other nonradioactively labelled substrates, the same assays conditions were used and the sugar components were separated and stained as described above.

*Preparation of GDP-*L*-fucose.*—The first step of the preparative synthesis of GDP-β-L-fucose is the conversion of GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose. The preparative enzyme reaction contained 165 μmol (100 mg) GDP-D-mannose, 50 mM Tris–HCl-buffer pH 7.5, 10 mM $MgCl_2$, crude extract of *E. coli* JM109 (DE3) pCAW21.1 with a GDP-D-mannose 4,6-dehydratase activity of 120 nkat in a final volume of 6 mL. This mixture was incubated at 37 °C for 60 min. The proteins were removed by boiling for 1 min and subsequent centrifugation at 10,000*g* for 30 min. The second step of conversion was started by the addition of 200 μmol NADPH and 120 nkat GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase-4-reductase (His-tag WcaG) to the supernatant. The mixture was incubated a second time at 37 °C for 60 min, and again boiled and centrifuged. The

course of each reaction to GDP-β-L-fucose was monitored by HPLC analysis with UV-detection (Beckman, Munich, Germany) at 260 nm. As the mobile phase, a phosphate-buffer (30 mM potassium phosphate pH 6.0; 5 mM tetrabutylammonium hydrogensulfate, 4% MeCN) and MeCN were used in combination with a reversed-phase column Eurospher ODS18, 5 μm, 250 × 4.6 mm (Knauer, Berlin, Germany).[27]

GDP-β-L-fucose was purified from $NADP^+$ and NADPH by preparative HPLC. The chromatography was performed with an Eurospher ODS18 (20 × 250 mm) column (Knauer, Berlin, Germany) using the same conditions described as above for the analytical chromatography. GDP-L-fucose-containing fractions were pooled and evaporated to a volume of 12 mL under diminished pressure (20 mbar) at 20–25 °C. The preparation was desalted by gel filtration on a Sephadex G-10 column (SR 25/100; Pharmacia, Freiburg, Germany), in a total volume of 398 mL and eluted at a flow rate of 1 mL/min. The GDP-β-L-fucose-containing fractions were pooled and applied to a membrane anion exchanger Q15 (Sartorius, Göttingen, Germany) and the GDP-β-L-fucose preparation was equilibrated against 150 mM NaCl to yield the Na^+ form. After another volume reduction by evaporation to a volume of about 12 mL and a further gel filtration (Sephadex G-10 column), the sodium GDP-β-L-fucose was lyophilised (Cryograph LCD-1, Christ, Osterrode, Germany) (78 mg, 78%).

^{1}H NMR (400 MHz, D_2O): δ 1.1 (d, H-6″, $^3J_{5''6''}$ 6.4 Hz); 3.55 (dd H-2″, $^3J_{2''3''}$ 11 Hz, $^3J_{1''2''}$ 8.1 Hz); 3.65 (dd, H-3″, $^3J_{3''4''}$ 3.5 Hz, $^3J_{2''3''}$ 11 Hz); 3.71 (m, H-4″, $^3J_{3''4''}$ 3.5 Hz); 3.75 (m, H-5″); 4.2 (m, 2 H, H-5a′H-5b′); 4.52 (m, H-4′); 4.59 (dd, H-3′); 4.8 (dd, H-2′, $^3J_{1'2'}$6.1 Hz, $^3J_{2'3'}$3.7 Hz); 4,91 (dd, H-1″, $^3J_{1''2''}$ 8.1 Hz, $^3J_{\text{P-2H1''}}$ 8.3 Hz); 5.9 (d, H-1′, $^3J_{1'2'}$6.1 Hz); 8,1 (s, H-1); ^{13}C NMR (100 MHz, D_2O): δ 17.5 C′-6″; 33.2 C-2′; 64.6 C-5′; 73.1 73.2 72.5 C-3″ C-4″ C-5′; 74.5 C-3″; 75.8 C-2″; 100.4 C-1″; 118.3 C-5; 139.6 C-1; 153.9 C-4; 156.2 C-2; 161.2 C-6; ^{31}P NMR (160 MHz, D_2O): δ −9.7 (P-1, $^2J_{PP}$ 19.9 Hz); −11.5 (P-2, $^2J_{pp}$ 19.9 Hz, $^3J_{\text{P-2H-1''}}$8.3 Hz).

2′-Fucosyllactose (α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose).—A solution containing GDP-L-fucose (35 mg, 57 μmol sodium salt), lactose (30 mg, 80 μmol), the GST-FucT2 protein (10 nkat) was incubated at 37 °C for 8 h in reaction buffer (10 mM $MnCl_2$, 100 mM NaCl in 50 mM Tris–HCl pH 7.5). Boiling and centrifugation removed the proteins. The mixture was purified by an anion-exchange column chromatography (Dowex 1 × 8 resin, mesh 200–400; Cl-form, Serva, 1 × 10 cm, water). The eluate was concentrated under diminished pressure and then the product was further purified by gel filtration on a column of Sephadex G-10 (2.5 × 100 cm, water; Pharmacia, Germany), equipped with a RI-detector (Knauer, Germany), to give 2′-fucosyllactose (18 mg, 65%). 2′-Fucosyllactose and other saccharides were analysed by means of HPAEC–PAD using a Dionex DX-500 system equipped with a PA10 column (Dionex, Sunnyvale, USA). Spectrometric data for α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose: ^{1}H NMR (400 MHz, D_2O): δ 5.27 (d, H-1″), 5.18 (d, $J_{1,2}$ 3,3 Hz, H-1α), 4.60 (d, $J_{1,2}$ 7.7 Hz, H-1β), 4.48 (d, $J_{1,2}$ 7.6 Hz, H-1′), 4.21 (m, $J_{5,6}$ 6.5 Hz, H-5″), 3.79 (d, $J_{2,3}$ 9.8 Hz, H-2″), 3.25 (dd, $J_{1,2}$ 7.7 Hz, H-2β), 1.19 (d, $J_{5,6}$ 6.5 Hz, H-6″); ^{13}C NMR (100 MHz, D_2O): δ 102.51 (C-1′), 101.59 (C-1″), 98.16 (C-1α), 94.08 (C-1β), 78.56 (C-4α), 78.36 (C-4β), 78.15 (C-3″), 77.48 (C-5″), 76.64 (C-3β), 76.56 (C-5β), 76.22 (C-3′), 75.87 (C-2″), 74.82 (C-4″), 73.94 (C-3β), 73.56 (C-2β), 71.93 (C-2α), 71.42 (C-5′), 71.40 (C-5α), 70.46 (C-2″), 69.16 (C-4′), 62.45 (C-6β), 62.35 (C-6α), 61.91 (C-6′), 17,49 (C-6″); ESIMS: m/z $[M + Na]^+$ 511.04.

Acknowledgements

We thank Roche Diagnostics, Penzberg, Germany, for financial support and for the preparation of the *H. pylori* DNA. We are grateful to Professor Dr J. Thiem and his coworkers for the synthesis of acceptor oligosaccharides used in this study. We also thank Y. Rockser and E. Sauerbier for technical assistance.

References

1. Fukuda, M. In *Alfred Benzon Symposium*; Bock, K.; Clausen, H., Eds. Complex Carbohydrates in Drug Research; Munksgaard: Copenhagen, 1994; pp. 353–365.
2. Liu, H.-W.; Thorsen, J. S. *Annu. Rev. Microbiol.* **1994**, *48*, 223–256.
3. McAuliffe, J.; Hindsgaul, O. *Chem. Ind.* **1997**, 170.
4. Ernst, B.; Oehrlein, R. *Glycoconjugate J.* **1999**, *16*, 161–170.
5. Becker, D. J.; Lowe, J. B. *Biochim. Biophys. Acta* **1999**, *1455*, 193–204.
6. Chan, N. W. C.; Stangier, K.; Sherburne, R.; Zaylor, D. E.; Zhang, Y.; Dovichi, J.; Palcic, M. M. *Glycobiology* **1995**, *5*, 683–688.
7. Wang, G.; Boulton, P. G.; Chan, N. W. C.; Palic, M. M.; Taylor, D. E. *Microbiology* **1999**, *145*, 3245–3253.
8. Rasko, D. A.; Wang, G.; Palcic, M. M.; Taylor, D. E. *J. Biol. Chem.* **2000**, *275*, 4988–4994.
9. Taylor, D. E.; Rasko, D. A; Sherburne, R.; Ho, C.; Jewel, L. D. *Gastroenterology* **1998**, *115*, 1113–1122.
10. Albermann, C.; Distler, J.; Piepersberg, W. *Glycobiology* **2000**, *10*, 875–881.
11. Schmidt, R. R.; Wegmann, B.; Jung, K. H. *Liebigs Ann. Chem.* **1991**, 121–124.
12. Gokhale, U.; Hindsgaul, O.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063–1071.
13. Elling, L.; Ritter, J. E.; Verseck, S. *Glycobiology* **1996**, *6*, 591–597.
14. Fey, S.; Elling, L.; Kragl, U. *Carbohydr. Res.* **1997**, *305*, 475–481.
15. Adrianopoulus, K.; Wang, L.; Reeves, P. R. *J. Bacteriol.* **1998**, *180*, 998–1001.
16. Sullivan, F. X.; Kumar, R.; Kriz, R.; Stahl, M.; Xu, G.-Y.; Rousse, J.; Chang, X.; Boodhoo, A.; Potvin, B.; Cumming, D. A. *J. Biol. Chem.* **1998**, *273*, 8193–8202.
17. Hanahan, D. *J. Mol. Biol.* **1983**, *166*, 557–580.
18. Miller, J. H *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory: New York, 1972.
19. Sambrook, J.; Fritsch, E. F.; Maniatias, T. *Molecular Cloning*: *A Laboratory Manual*; Cold Spring Harbor Laboratory: New York, 1989.
20. Stevenson, G.; Hobbs, M.; Andrianopoulos, K.; Reeves, P. R. *J. Bacteriol.* **1996**, *178*, 4885–4893.
21. Laemmli, U. K. *Nature* **1970**, *277*, 680–685.
22. Tomb, J. F.; White, O.; Kerlavage, A. R.; Clayton, R. A.; Sutton, G. G.; Fleischmann, R. D.; Ketchum, K. A.; Klenk, H. P.; Gill, S.; Dougherty, B. A.; Nelson, K.; Quackenbush, J.; Zhou, L.; Kirkness, E. F.; Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H. G.; Glodek, A.; McKenney, K.; Fitzegerald, L. M.; Lee, N.; Adams, M. D.; Hickey, E. K.; Berg, D. E.; Gocayne, J. D.; Utterback, T. R.; Peterson, J. D.; Kelley, J. M.; Karp, P. D.; Smith, H. O.; Fraser, C. M.; Venter, J. C. *Nature* **1997**, *388*, 539–547.
23. Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463–5467.
24. Okazaki, R.; Okazaki, T.; Strominger, J. T.; Michelson, A. M. *J. Biol. Chem.* **1962**, *237*, 3014–3026.
25. Kornfeld, R. H.; Ginsburg, V. *Biochim. Biophys. Acta* **1966**, *117*, 79–87.
26. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
27. Payne, S. H.; Ames, B. N. *Anal. Biochem.* **1982**, *123*, 151–161.
28. Koizumi, S.; Endo, T.; Tabata, K.; Nagano, H.; Onishi, J.; Ozaki, A. *J. Ind. Microbiol. Biotechnol.* **2000**, *25*, 213–217.